REMARKS/ARGUMENTS

Claims 2-17 and 29-32 were previously cancelled. Claims 23-28 have been withdrawn as the result of an earlier restriction requirement. Claims 1, and 18-22 remain pending in this application.

At the Examiner's request, applicant confirms that the claims were commonly owned at the time that the invention covered therein was made.

Objection under 35 U.S.C. 103

The Examiner has rejected claim 1 under 35 USC 103(a) as obvious in view of Wakarchuk et al. and Sung et al. The Examiner contends that it would have been obvious to a person of skill in the art to obtain a xylanase with at least one disulfide bond and a basic amino acid at position 162, given the disclosures of Wakarchuk et al. and Sung et al. The Examiner further alleges that such a xylanase "would inherently have the increased thermostable and alkilophilic characteristics". Applicant respectfully disagrees with the Examiner's rejection for the following reasons.

Claim 1 is directed to a modified Family 11 xylanase comprising a basic amino acid at position 162 (determined from sequence alignment of said modified xylanase with *Trichoderma reesei* xylanase II amino acid sequence) and at least one intramolecular disulfide bond. The modified xylanase is further characterized as exhibiting at least 40% of optimal activity from about pH 3.5 to about pH 6.0, and from about 40 to about 60°C, and as being thermostable.

The advantage of a having both a basic amino acid at position 162 and at least one intramolecular disulfide bond is identified for the first time in the present application. For example, as shown in Figures 3 or 4B, xylanase mutants that consist of only an intramolecular disulfide bond

(e.g., TrX-DS1; with no basic amino acid at position 162), or a mutant that consists of only a basic amino acid at position 162 (e.g. TrX-162H, with no intramolecular disulfide bond), do not exhibit the characteristic of thermostability as required in claim 1. However, the combination of these mutations (e.g., TrX-162-DS1, TrX-162H-DS4 or trX-162H-DS8), results in increased thermostability.

Wakarchuk teaches increased thermostability of *B. circulans* xylanase mutants by introduction of disulfide bridges in the enzyme, but which do not comprise a basic amino acid at position 162. The results of Wakarchuk show that some disulfide bonds may have different effects on thermostability. For example, the TS1 and TS6 mutants show improved thermostability (Figure 4); however, the TS2 mutant is less thermostable than TS1 despite the similarities of their environments (page 1382, sentence bridging columns 1-2; Figure 4). There is no suggestion or teaching within Wakarchuk of producing a xylanase comprising both a disulfide bridge and a basic amino acid at position 162. The references of Sung et al. disclose a number of xylanase mutants, one of which comprises a basic amino acid at position 162 (NI-TX1; comprising TvX3-190: recombinant *T. reseei* xylanase with Ala-1, Ser-2, Gly-4, Phe-9, Thr-65, Thr-143, and a Q162H mutation). However, none of the mutants disclosed in Sung have disulfide bridges. The NI-TX1 mutant showed the same activity profile as the TvX(3-190), both of which showed much less activity than the natural enzyme (see col 29, lines 44-45 and 52-53; Figure 5). In addition, the NI-TX1 mutant shows no improvement in thermostability over the natural enzyme (column 34, lines 42-46; Figure 10), and hence there would be no motivation to further explore this mutation as is done in the present

invention. There is no suggestion or teaching within Sung of producing a xylanase comprising a basic amino acid at position 162 and a disulfide bridge as defined in claim 1.

In order for an invention to be "obvious" within the meaning of 35 USC § 103 in view of a combination of references, the combination must be suggested in the prior art or by the very nature of the cited references. In re Geiger, 815 F.2d 686 2 U.S.P.Q. 2d 1276 (Fed. Cir. 1987); Uniroyal v. Rudkin-Wiley, 837 F.2d 1044, 5 U.S.P.Q. 2d 1434 (Fed.Cir.1988); Interconnect Planning Corp. v. Feil, 774 F.2d 1132, 227 U.S.P.Q. 543 (Fed. Cir. 1985). The mere fact that a prior device can be modified to produce the claimed invention is not a proper basis for an obviousness rejection unless the art suggests the desirability of such a modification. In re Gordon, 733 F.2d 900, 221 U.S.P.Q. 1125 (Fed. Cir. 1984). A showing that two or more references are related to a similar subject matter does not, by itself, suggest the possibility of desirability of a combination of those references. In re Levitt, 11 U.S.P.Q. 2d 1315 (Fed. Cir. 1989).

Applicant submits that there is no teaching or suggestion in Wakarchuk and Sung to combine disulfide bonds and a basic amino acid at position 162. Furthermore, it is submitted that neither Wakarchuk nor Sung teach or suggest that the combination of these mutations would lead to increased thermophilicity or thermostability.

Applicant further submits that it is well known in the art that the effect of engineered mutations on protein stability cannot be generalize or readily predicted. In fact, this view is expressed in Sung (column 4, line 64 to column 5, line 4):

> The effects of different mutations on enzyme characteristics, including thermophilicity and alkalophilicity, are often unpredictable. Generally, only a tiny fraction of all

possible modifications, if any, provide significant benefit. Therefore, setting out to improve the properties of a protein by protein engineering is a difficult venture, and the limited success to date with Family 11 xylanases reflects this.

A similar statement is made in Wakarchuk et al., at page 1383, first sentence: "The effects of disulfide bonds on the thermostability of the proteins cannot always be predicted". Therefore, the scientific facts contradict the Examiner's statement that a xylanase with a disulfide bond and a basic amino acid at position 162 would inherently be thermostable.

The present application shows that the thermostability of a xylanase having the Q162H mutation (TrX-Q162H) is lower than that of the wild-type enzyme (TrX), and that a xylanase having one disulfide bond (TrX-DS1) showed somewhat improved thermostability over TrX (page 33, lines 12-15; Figure 3). However, the TrX-Q162H-DS1 mutant (one disulfide bond and the Q162H mutation) shows substantially increased thermostability compared to TrX, TrX-Q162H, and TrX-DS1 (page 33, lines 15-18; Figure 3). Furthermore, the increase in thermostability is observed in several xylanases comprising the double mutation, for example, Trx-162H-DS1; TrX-162H-DS4; TrX-162H-DS8, as shown in figures 3, 4B, and 5.

Applicant submits that a person of skill in the art could not have predicted that the engineered xylanase would be thermostable, nor could they have predicted the substantial increase in thermostability obtained by adding a disulfide bond to TrX-Q162H. Such a large increase in thermostability is necessarily unobvious.

In light of the above comments and amendments, Applicant respectfully requests the withdrawal the rejection under 35 USC 103(a) against claim 1.

It is respectfully submitted that the above-identified application is now in a condition for allowance and favourable reconsideration and prompt allowance of these claims are respectfully requested. Should the Examiner believe that anything further is desirable in order to place the application in better condition for allowance, the Examiner is invited to contact the applicant's undersigned attorney at the telephone number listed below.

It is believed that no fees are due at this time. However, the Commissioner is hereby authorized to charge any additional fees that may be required, or credit any overpayment to Deposit Account No. 14-0629.

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450 Alexandria, Virginia 22313-1450 on the date shown below.

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11-8-04 — Date